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Title: Learning about synaptic GluA3

Zhengping Jia[1,2] and Graham L. Collingridge[1,3,4]

[1]Department of Physiology, Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada M5S 1A8

[2]Neurosciences & Mental Health, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, Canada M5G 1X8

[3]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, 600 University Avenue Toronto, Ontario, Canada M5G 1X5

[4]Centre for Synaptic Plasticity, Department of Physiology, Pharmacology & Neuroscience, Dorothy Hodgkin Building, University of Bristol, Bristol, BS1 3NY, UK

Corresponding author: Graham Collingridge at glcollingridge@gmail.com and Zhengping Jia at zhengping.jia@sickkids.ca

Abstract

In this issue of Neuron, Gutierrez-Castellanos et al. (2016) reveals a critical role for the AMPA receptor subunit GluA3 in cerebellar synaptic plasticity and motor learning in mice.

Long-term potentiation (LTP) and long-term depression (LTD) at glutamatergic synapses are extensively studied forms of long-lasting synaptic plasticity that are widely regarded as key mechanisms for learning and memory, and deficits in LTP and LTD are linked to many neurological and mental disorders, including intellectual disability, autism and Alzheimer disease (Henley and Wilkinson, 2016). Thus, the mechanisms governing these forms of plasticity are a subject of intensive investigation. At the core of this research is the involvement of the AMPA subtype of ionotropic glutamate receptors (AMPARs), because these receptors are the principal mediator of fast excitatory synaptic transmission in the CNS and since considerable evidence has accumulated that changes in either the properties or the number of AMPARs are critically involved in the expression of major forms of both LTP and LTD at many central synapses. However, the extent to which these receptors, and their composite subunits, mediate these changes remains controversial (Huganir and Nicoll, 2013; Henley and Wilkinson, 2016).

AMPARs are heteromeric complexes consisting of various combination of four different subunits (GluA1-4). The mechanisms of LTP and LTD have been mainly studied at the excitatory synapses between CA3 and CA1 pyramidal neurons in the hippocampus. Here, a large body of evidence links GluA1-containing AMPARs with LTP and GluA2-containing AMPARs with LTD. In contrast, little is known about the possible role of GluA3-containing AMPARs in synaptic plasticity. Global genetic deletion of GluA3 produces no clear effect on LTP or LTD in the hippocampus, suggesting that this subunit may play little or no role (Meng et al., 2003). However, it is knows that GluA3 is present at many central synapses and contribute to the formation of functional AMPARs, and that mutations in the GluA3 gene are linked to a number of brain disorders (e.g., Wu et al., 2007), suggesting a key role for this subunit in brain

function. However, whether the GluA3 subunit regulates synaptic plasticity and behavior remains elusive. In this issue of Neuron, Gutierrez-Castellanos et al. addressed this issue by investigating the role of GluA3 in cerebellar synaptic properties and cerebellum-dependent motor learning using GluA3 KO mice. Compared to GluA1 KO (Zamanillo et al., 1999) and GluA2 KO (Jia et al, 1996) mice, which show deficits in both basal synaptic function and behavior, including severe motor deficits in the case of GluA2, the GluA3 KO mice appear more normal (Meng et al., 2003), providing a useful model for studying their role in CNS function.

First, the authors demonstrated that GluA3 KO mice were severely impaired in the vestibulo-ocular reflex (VOR) phase-reversal adaptation, a well established cerebellum-dependent motor learning paradigm, in which the mice learns to shift the phase of their VOR following sinusoidal visuovestibular mismatch stimulation (Gao et al., 2012). Interestingly, GluA1 KO mice were not altered in this test. This finding is rather surprising given that GluA1 is actually more highly expressed than GluA3 in the cerebellum and that much of early work, primarily from the hippocampus, indicates the critical involvement of GluA1, but not GluA3, in declarative memory (Kessels and Malinow, 2009). These results suggest an essential role of GluA3 in cerebellum-dependent behavior.

To determine the cellular basis for the motor learning defects in GluA3 KO mice, the authors then examined synaptic function in Purkinje cells (PCs). Early studies show that synaptic plasticity of PCs is important for motor learning (Gao et al., 2012). They recorded spontaneous miniature excitatory postsynaptic currents (mEPSCs) of PCs in cerebellar slices and found that both the amplitude and frequency of mEPSCs were significantly lower in GluA3 KO, but not

GluA1 KO, compared to wild-type (WT) control. In GluA1/3 double KO mice, mEPSC events were virtually absent. These results suggest that the majority of synaptic responses in PCs are derived from either GluA1- or GluA3-containing AMPARs. To examine synaptic plasticity, LTD and LTP were then compared using evoked EPSCs at the parallel fiber (PF) to PC (PF-PC) synapse. While the magnitude of LTD was not altered in GluA1 KO or GluA3 KO mice, LTP was abolished in GluA3 KO mice, but remained intact in GluA1 KO mice. Therefore, while both GluA1 and 3 contribute to basal synaptic transmission, GluA3 is uniquely indispensable for LTP at the PF-PC synapse. Of note, previous studies have established that GluA2 is critical for LTD at this synapse (Gao et al., 2012).

To elucidate the molecular mechanism underlying GluA3-dependent LTP at the PF-PC synapse, the authors then switched to a form of chemically induced synaptic potentiation by application of the adenylyl cyclase activator forskolin (FSK). Again this form of LTP was abolished in GluA3 KO, but remained intact in GluA1 KO mice, suggesting that GluA3-mediated potentiation is downstream of cAMP signaling. In the next series of experiments, the authors combined imaging, single channel conductance analysis and pharmacological inhibitors to demonstrate that the GluA3-mediated synaptic potentiation by FSK was independent of synaptic insertion or lateral diffusion of GluA3-containing AMPARs, but involved an increase in the channel properties of the receptors. Specifically they found an increase in the time that the channels occupied higher conductance substates leading to an overall increase in single channel conductance. Interestingly, this FSK induced synaptic plasticity did not require PKA, but depended on Epac, an exchange factor directly activated by cAMP. Activation of Epac was also sufficient to induce GluA3 dependent synaptic potentiation.

Is the FSK induced synaptic potentiation relevant to LTP at the PF-PC synapse and motor learning? To test this, the authors examined the role of Epac on LTP induced by tetanic stimulation. As predicted, the LTP was blocked by an Epac inhibitor. In addition, Epac activation enhanced basal response and occluded tetanically-induced LTP. Consistent with the effect on LTP, inhibition of Epac in WT mice significantly impaired motor learning in the VOR phase-reversal adaptation test, similar to the deficits observed in GluA3 KO mice.

Finally, to confirm that GluA3 in the PCs is actually responsible for the GluA3-mediated LTP and motor learning, the authors utilized a mouse strain where GluA3 was deleted specifically in the PCs using a Cre-LoxP method. Similar to the global GluA3 KO mice, these PC-specific KO mice also exhibited defective learning in the VOR phase-reversal adaptation test, indicating the critical importance of GluA3 in PCs for motor learning.

The study by Gutierrez-Castellanos et al. is of considerable significance for a number of reasons. First, it reveals for the first time a major role for GluA3 in both basal synaptic function and synaptic plasticity in cerebellar PCs. This is in marked contrast to CA1 pyramidal neurons where GluA3 appears to contribute little to basal synaptic transmission, LTP or LTD (Meng et al., 2003). Rather, GluA1 is the dominant subunit for LTP and GluA2 for LTD. Second, this study indicates that GluA3-mediated LTP is associated with changes in the channel properties, but not receptor trafficking. Previous work has identified an increase in AMPAR single channel conductance as an underlying mechanism of LTP at CA1 synapses (Benke et al., 1998), but whether this is due to a modification of the properties of AMPARs already at the synapse or the

synaptic insertion of higher conductance AMPARs is unknown. At the PF-PC synapse, the effects appears to be due to a direct modification of AMPARs to increase single channel conductance. Third, GluA3-mediated channel properties changes require cAMP, but surprisingly is independent of PKA. This is again distinct from the CA1 synapse where direct phosphorylation of GluA1 is important to alter the channel conductance and/or trafficking of AMPARs (Huganir and Nicoll, 2013). Interestingly, the GluA3-dependent LTP requires Epac but how this protein modulates the channel properties of GluA3 containing AMPARs remains to be investigated. Finally the study reveals that GluA3, not GluA1, plays a key role in motor learning, in contrast to the acquisition of declarative memories such as fear memory where GluA1 is essential (Kessels and Malinow, 2009).

In summary, the study by Gutierrez-Castellanos and colleagues has provided compelling evidence that GluA3 plays an essential role in LTP at the PF-PC synapse and motor learning. This GluA3-dependent synaptic plasticity is distinct from the GluA1-driven LTP in the hippocampus in that it involves exclusively enhanced channel conductance, but not trafficking, of AMPARs. It would be interesting to know whether this form of plasticity also exists in other regions of the brain. Another outstanding question is how the activation of cAMP-Epac signaling leads to changes in the channel properties of AMPARs. It would also be interesting to know whether this mechanism interacts with GluA2-mediated receptor trafficking shown to occurs during LTD at this synapse. Cleary there is much still to do, but the findings of Gutierrez-Castellanos et al., adds to the diversity of mechanisms of synaptic plasticity that underlies forms of learning and memory in the CNS.

References

Benke TA, Luthi A, Isaac JT, Collingridge GL (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. Nature 393, 793-797.

Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna FA, Velumian A, MacDonald J, Carlen P, et al (1996) Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 17:945-956

Gao Z, van Beugen BJ, De Zeeuw CI (2012). Distributed synergistic plasticity and cerebellar learning. Nat. Rev. Neurosci. 13, 619-635.

Gutierrez-Castellanos N., Da Silva-Matos CM, Zhou K, Canto CB, Renner MC, Koene LMC, Ozyildirim O,Sprengel R, Helmut W, Kessels HW, De Zeeuw CI (2016) Motor Learning Requires Purkinje Cell Synaptic Potentiation through Activation of AMPA-Receptor subunit GluA3. Neuron, this issue

Henley JM, Wilkinson KA. (2016) Synaptic AMPA receptor composition in development, plasticity and disease. Nat Rev Neurosci.17:337-50.

Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. Neuron 80:704-17.

Kessels HW, Malinow R (2009) Synaptic AMPA receptor plasticity and behavior. Neuron 61, 340-350.

Meng Y, Zhang Y, Jia Z (2003) Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. Neuron 39, 163-176.

Wu Y, Arai AC, Rumbaugh G, Srivastava AK, Turner G, Hayashi T, Suzuki E, Jiang Y, Zhang L, Rodriguez J, Boyle J, Tarpey P, Raymond FL, Nevelsteen J, Froyen G, Stratton M, Futreal A, Gecz J, Stevenson R, Schwartz CE, Valle D, Huganir RL, Wang T. (2007) Mutations in ionotropic AMPA receptor 3 alter channel properties and are associated with moderate cognitive impairment in humans. Proc Natl Acad Sci U S A. 104:18163-8.

Zamanillo D, Sprengel R, Hvalby O, Jensen V, Burnashev N, Rozov A, Kaiser KM, Köster HJ, Borchardt T, Worley P, Lübke J, Frotscher M, Kelly PH, Sommer B, Andersen P, Seeburg PH, Sakmann B (1999) Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. Science 284:1805–11.

Figure Legend

Figure 1. GluA3-mediated LTP at the PF-PC synapse. Under basal condition, both GluA1/2 and GluA2/3 receptor types contribute to synaptic transmission. Upon LTP induction, the activation of cAMP/Epac signaling leads to increased single channel conductance of GluA3-containing receptors and thereby enhancing synaptic transmission. The numbers of GluA1/2 and GluA2/3 AMPARs are not changed by LTP induction.

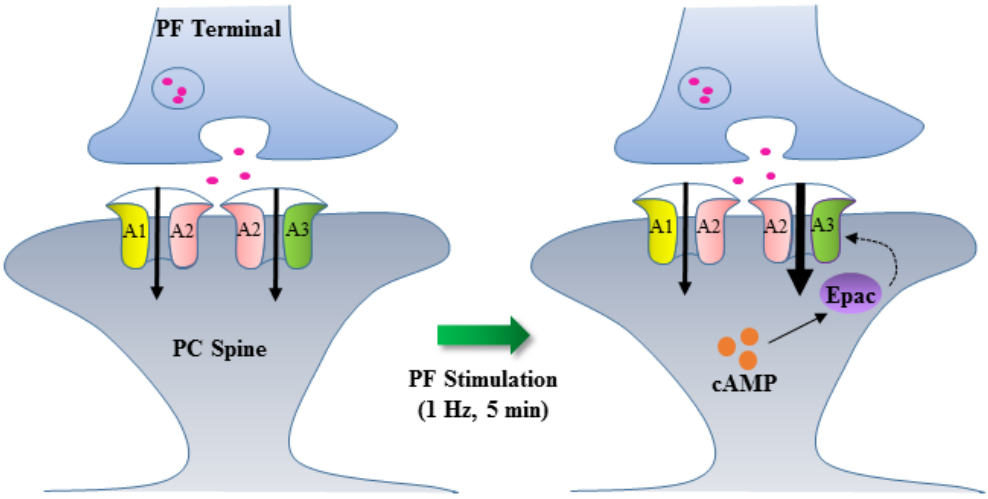

Figure 1. GluA3-mediated LTP at PF-PC synapses. Under basal condition, both GluA1/2 and GluA2/3 receptor types contribute to synaptic transmission. Upon LTP induction, the activation of cAMP/Epac signaling leads to increased channel conductance of GluA3-containing receptors and thereby enhancing synaptic transmission. The numbers of GluA1/2 and GluA2/3 AMPARs are not changed during LTP.